

CLAIMS:

1. A human feeder cell layer which supports the derivation of ES cells in a substantially undifferentiated state said feeder cell layer comprising cells selected from the group including human adult, fetal or embryonic cells or a combination thereof.
2. A human feeder cell layer which supports the culture of ES cells in a substantially undifferentiated state said feeder cell layer comprising cells selected from the group including human adult, fetal or embryonic cells or a combination thereof.
3. A human feeder cell layer according to claim 1 or 2 wherein the human adult cell is selected from the group including human fibroblast cells, human adult skin and human adult muscle fibroblasts and adult epithelial cells or a combination thereof.
4. A human feeder cell layer according to claim 3 wherein the human adult cell is a human fibroblast cell.
4. A human feeder cell layer according to claim 3 or 4 wherein the human fibroblast cell is a human adult fallopian tubal (HAFT) fibroblast cell.
5. A human feeder cell layer according to claim 3 wherein the human adult cell is a human skin cell.
6. A human feeder cell layer according to claim 3 wherein the human adult cell is a human muscle cell.
- 30 7. A human feeder cell layer according to claim 3 wherein the human adult cell is a human adult epithelial cell.
8. A human feeder cell layer according to claim 3 or 7 wherein the human adult epithelial cell is a human oviductal epithelial cell.

9. A human feeder cell layer according to claim 1 or 2 wherein the human fetal cell is a human fetal muscle (HFM) or human fetal skin (HFS) cell or combination thereof.
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10. A human feeder cell layer according to claim 9 wherein the human fetal cell is a HFM cell.
11. A human feeder cell layer according to claim 9 wherein the human fetal cell is a HFS cell.
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12. A human feeder cell layer according to claim 1 or 2 wherein the human embryonic cell is a human embryonic muscle (HEM) or human embryonic skin cell or combination thereof.
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13. A human feeder cell layer according to claim 12 wherein the human embryonic cell is a HEM cell.
14. A human feeder cell layer according to claim 12 wherein the human embryonic cell is a human embryonic skin cell.
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15. A human feeder cell layer according to any one of claims 1 to 14, which is first established in a primary culture in the presence of HFE medium.
- 25 16. A human feeder cell layer according to anyone of claims 1 or 15 wherein the feeder layer is propagated in the presence of a HM medium.
17. A human feeder cell layer according to any one of claims 1 to 16 comprising fibroblast cell line Detroit 551 (ATCC NO CCL-110).
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18. A human feeder cell layer according to any one of claims 1 to 16 comprising cell line MRC-5 having Accession Number ATCC No X-55 or ATCC No CCL-171.

19. A human feeder layer according to any one of claims 1 to 16 comprising cell line WI-38 having Accession Number ATCC NO CCL-75 or ATCC NO CCL-75.1.
- 5 20. A method of deriving an embryonic stem (ES) cell line in a substantially undifferentiated state from an ES cell population said method comprising: obtaining an ES cell population comprising undifferentiated ES cells; and culturing the undifferentiated ES cells on a cell support matrix in the presence of soluble factors derived from human feeder cells or equivalents 10 thereof.
- 15 21. A method according to claim 20 wherein deriving an ES cell line is selected from the group including creating an ES cell line from a source of ES cells and wherein the ES cells are previously uncultured cells; extending propagation or culturing time of an ES cell line wherein the ES cell line is an established cell line; and propagating an established ES cell line.
- 20 22. A method according to claim 20 or 21 wherein the deriving of the ES cell line includes propagating an ES cell line.
- 25 23. A method according to any one of claims 20 to 22 wherein the ES cell population is derived from a source selected from the group including an embryo, blastocyst, inner cell mass (ICM) cells, and a culture of ES cells which have not differentiated.
- 30 24. A method according to claim 23 wherein the source is from a blastocyst.
25. A method according to any one of claims 20 to 24 wherein the soluble factors are derived from human feeder cells selected from the group including human adult, fetal or embryonic cells or a combination thereof.
- 30 26. A method according to claim 25 wherein the human adult cell is selected from the group including human fibroblast cells, human adult skin and human adult muscle fibroblasts and adult epithelial cells or a combination thereof.

27. A method according to claim 26 wherein the human adult cell is a human fibroblast cell.
- 5 28. A method according to claim 26 or 27 wherein the human fibroblast cell is a human adult fallopian tubal (HAFT) fibroblast cell.
29. A method according to claim 26 wherein the human adult cell is a human skin cell.
- 10 30. A method according to claim 26 wherein the human adult cell is a human muscle cell.
31. A method according to claim 26 wherein the human adult cell is a human adult epithelial cell.
- 15 32. A method according to claim 26 wherein the human adult cell is a human oviductal epithelial fibroblast.
- 20 33. A method according to claim 25 wherein the human fetal cell is a human fetal muscle (HFM) or human fetal skin (HFS) cell or combination thereof.
34. A method according to claim 33 wherein the human fetal cell is a HFM cell.
- 25 35. A method according to claim 33 wherein the human fetal cell is a HFS cell.
36. A method according to claim 25 wherein the human embryonic cell is a human embryonic muscle (HEM) or human embryonic skin cell or combination thereof.
- 30 37. A method according to claim 36 wherein the human embryonic cell is a HEM cell.

38. A method according to claim 36 wherein the human embryonic cell is a human embryonic skin cell.
- 5 39. A method according to any one of claims 20 to 38 wherein the human feeder cells are cultured in the presence of a medium selected from the group including HES, KO, HF, HES-HS, KO-HS, and HF-HS as hereinbefore described.
- 10 40. A method according to claim 39 wherein the medium is HES-HS or KO-HS.
41. A method according to claim 40 wherein the medium is KO-HS.
- 15 42. A method according to any one of claims 20 to 41 wherein the cell support matrix is a non-cellular cell support matrix selected from the group including Collagen I, Collagen IV, human extracellular matrix or Matrigel™ or a combination thereof.
- 20 43. A method according to any one of claims 20 to 42 wherein the cell support matrix comprises Collagen I or Type I Collagen.
44. A method according to any one of claims 20 to 41 wherein the cell support matrix comprises a human feeder cell layer according to any one of 25 claims 1 to 19.
45. A method according to any one of claims 20 to 44 wherein the ES cells are cultured in the presence of a medium selected from the group including HES, KO, HES-HS, KO-HS and HF-HS as hereinbefore described.
- 30 46. A method according to claim 45 wherein the medium is KO-HS.

47. A method according to any one of claims 20 to 46 wherein the feeder cells are first established in primary cultures in the presence of HFE medium, as hereinbefore described.

5 48. A method according to any one of claims 20 to 47 wherein the feeder cells are propagated in the presence of a HM medium prior to culture with ES cells, as hereinbefore described.

10 49. A method according to any one of claims 20 to 48 wherein the human feeder cell is the fibroblast cell line Detroit 551 (ATCC NO CCL-110).

50. A method according to any one of claims 20 to 48 wherein the human feeder cell is the cell line MRC-5 having Accession Number ATCC No X-55 or ATCC No CCL 171.

15 51. A method according to any one of claims 20 to 48 wherein the human feeder cell is the cell line WI-38 having Accession Number ATCC-CCL-75 or ATCC-CCL-75.1.

20 52. A method according to any one of claims 20 to 51 wherein the ES cell line is cultured in the absence of LIF.

53. A cellular composition comprising proliferating undifferentiated ES cells and wherein the cell composition comprises the propagated or derived ES cells prepared by the methods according to any one of claims 20 to 52.

25 54. An undifferentiated ES cell line prepared by a method according to any one of claims 20 to 52.

30 55. A cell culture system for deriving and culturing ES cells in a substantially undifferentiated state, said culture system including:
a cell support matrix; and

a cell culture medium for providing soluble factors derived from a human feeder cell selected from the group including a human adult, fetal or embryonic cell.

- 5 56. A cell culture system according to claim 55 wherein the human adult cell is selected from the group including human adult fallopian tubal (HAFT) fibroblast cells, human adult skin and human adult muscle fibroblasts and adult epithelial cells or a combination thereof.
- 10 57. A cell culture system according to claim 56 wherein the human adult cell is a human fibroblast cell.
58. A cell culture system according to claim 56 or 57 wherein the human adult cell is a human adult fallopian tubal (HAFT) fibroblast cell.
- 15 59. A cell culture system according to claim 56 wherein the human adult cell is a human skin cell.
60. A cell culture system according to claim 56 wherein the human adult cell 20 is a human muscle cell.
61. A cell culture system according to claim 56 wherein the human adult cell is a human adult epithelial cell.
- 25 62. A cell culture system according to claim 56 wherein the human epithelial adult cell is a human oviductal epithelial cell.
63. A cell culture system according to claim 55 wherein the human fetal cell 30 is a human fetal muscle (HFM) or human fetal skin (HFS) cell or combination thereof.
64. A cell culture system according to claim 63 wherein the human fetal cell is a HFM cell.

65. A cell culture system according to claim 63 wherein the human fetal cell is a HFS cell.
66. A cell culture system according to claim 55 wherein the human embryonic cell is a human embryonic muscle (HEM) or human embryonic skin cell or combination thereof.
67. A cell culture system according to claim 66 wherein the human embryonic cell is a HEM cell.
68. A cell culture system according to claim 66 wherein the human embryonic cell is a human embryonic skin cell.
69. A cell culture system according to claim 55 wherein the cell support matrix comprises Collagen I or matrigel or a combination thereof.
70. A cell culture system according to claim 69 wherein the cell support matrix comprises Collagen I.
71. A cell culture system according to any one of claims 55 to 70 wherein the cell culture medium is a conditioned medium including soluble factors derived from a human feeder cell layer.
72. A cell culture system according to claim 55 to 68 wherein the cell support matrix comprises a human feeder cell layer according to any one of claims 1 to 14.
73. A cell culture system according to any one of claims 55 to 72 wherein the culture medium is selected from the group including HES, KO, HES-HS, and KO-HS.
74. A cell culture system according to claim 73 wherein the medium is KO-HS.

75. A conditioned medium for deriving and culturing an ES cell line in a substantially undifferentiated state said medium prepared by a method including:

5 obtaining a feeder cell layer according to any one of claims 1 to 19;

 culturing the feeder cells in the presence of a medium selected from the group including HES, KO, HES-HS, KO-HS, HFE, HM, HF or HF-HS; and

 separating the medium from the cells to obtain conditioned medium.

76. A conditioned medium according to claim 75 wherein the human feeder

10 cell layer comprises adult skin cells.

77. A conditioned medium according to claim 76 wherein the human feeder cell layer comprises HFM cells.

15 78. A conditioned medium according to any one of claims 75 to 77 wherein the medium comprises KO-HS.